RSC Advances

This article can be cited before page numbers have been issued, to do this please use: K. H. Kumar, H. K. VIVEK, S. Rangappa, J. Fuchs, B. Kumar, B. C. Krishna, L. M. Mervin, P. BABU SHUBHA, S. Basappa, S. NanjundaSwamy, A. Bender and K. S. Rangappa, *RSC Adv.*, 2015, DOI: 10.1039/C5RA13085A.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

View Article Online
DOI: 10.1039/C5RA13085A

MOLPRINT 2D-based identification and synthesis of novel chromene based small molecules that target PLA2: Validation through chemo - and bioinformatics approach

Hosadurga K Keerthy ,[1] Hamse K Vivek,[2] Shobith Rangappa,[3] Julian E. Fuchs,[4] Hanumantharayappa Bharathkumar[1], Krishna C. Bulusu,[4] Lewis H. Mervin,[4] Babu S Priya,[5] Basappa,[1,*] Nanjuda Swamy S,[2,*] Andreas Bender,[4,*] Kanchugarakoppal S. Rangappa[5,*]

[1]Laboratory of Chemical Biology, Department of Chemistry, Bangalore University, Palace Road, Bangalore-560001, India; [2]Department of Biotechnology, Sri Jayachamarajendra College of Engineering, JSS Technical Institutions Campus, Mysore, 570 006, Karnataka, India; [3]Frontier Research Centre for Post-genome Science and Technology Hokkaido University, Japan; [4]Centre for Molecular Informatics, Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, United Kingdom; [5]Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore-570 006, India;

***Corresponding Authors**

Dr. Basappa
Department of Chemistry
Central College
Palace Road
Bangalore University
Bangaluru-560001
Karnataka, INDIA
Phone: +91-80-22961346
Email: salundibasappa@gmail.com

View Article Online
DOI: 10.1039/C5RA13085A

ABSTRACT:

Phospholipase A2 (PLA2) was known to regulate inflammation and hence it was considered as a validated drug-target by medicinal chemists. In this report, we have identified and considered highly ranked ligand of ZINC-drug-like compounds database, which targets PLA2 *via* MOLPRINT-2D based chemoinformatic drug-design approach. The computationally predicted lead molecule was found to contain the core moiety of chromene ring, which was well known for its varied biological properties. Here, a novel and efficient retro-synthetic protocol for the synthesis of highly substituted chromene libraries was made. One-pot synthesis of chromene was carried out by using different aromatic primary alcohols, malanonitrile and 4-hydroxy coumarin in the presence of mild oxidant mixture called $T_3P^{®}$-DMSO, followed by Suzuki coupling reaction to obtain the lead molecules. All the tested compounds of chromene series displayed the inhibition of the venom PLA2 with a range from 12 to 68 μM. Among the tested compounds, 2-amino-4-(2'-methyl-[1,1'-biphenyl]-4-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (**7b)** showed maximum inhibitory efficacy against venom PLA2 with an IC_{50} value of 12.5 μM. Furthermore, the designed PLA2 ligands bound to the active site of venom PLA2, whose binding affinity was comparable to nimesulide, indicate the chromene moiety containing ligands could be novel lead-structure that serves as anti-inflammatory agents.

Keywords**:** Retro-synthesis, 2-amino chromene-3-carbonitrile, MOLPRINT 2D, Snake venom PLA2.

View Article Online
DOI: 10.1039/C5RA13085A

INTRODUCTION

Phospholipase A2 (PLA2) was the most-studied membrane-bound enzyme with a molecular weight of 14 kDa[1-3]. It was a Ca^{2+}-dependent and disulphide-rich enzyme, and mostly present in mammalian tissues and in the venoms of insects and snakes[4]. It hydrolyzes the phospholipids from cellular membranes and lipoproteins at the sn-2 position releasing lysophospholipids and free fatty acids[5]. PLA2s are known to aid the production of eicosanoids, prostaglandins, leukotrienes and platelet-activating factors (PAF), which exert hormone-like function orchestrating various physiological events at lower concentrations [2]. However, higher levels may induce serious pathological conditions such as inflammation, arthritis, atherosclerosis and sepsis. Hence, PLA2 along with cyclooxygenases and lipoxygenases regulate inflammation and associated inflammatory diseases. Among the secretary PLA2s, group IIA was known to play a key role in both acute and chronic inflammation, which was regulated by multiple intracellular signalling cascades[6]. Besides regulation of human diseases, snake venom pathology was largely attributed to the presence of PLA2. Snake venoms have been demonstrated to be a complex mixture of PLA2, matrix metalloproteinase, hyaluronidases and other toxic non-enzymatic peptides[7]. The combined action of these enzymatic and non-enzymatic venom components are known to induce proteolysis, haemorrhage, necrosis, altered haemostasis, shock and several other neurological dysfunctions [8]. Among them, venom-induced necrosis, edema and anticoagulation are directly accredited to the myotoxic PLA2 and other myotoxins present in the venom.

In view of this, inhibition of PLA2 has been considered as a prime target in the management of inflammatory diseases and snakebites. Thus, the aim of research in this field was to identify safe and effective PLA2 inhibitors. The higher structural similarity

View Article Online
DOI: 10.1039/C5RA13085A

between the snake venom sPLA2 and of humans suggests using snake venom PLA2 inhibitors to design novel drugs aiming human inflammatory diseases and vice versa [9, 10]. Also, the active site of sPLA2 was composed of substrate binding hydrophobic region and substrate cleaving hydrophilic region, hence it was further requirement that a PLA2 inhibitor must bear hydrophobic and hydrophilic moieties in it[11]. To date, several synthetic[12] and natural[13] inhibitors have been reported as PLA2 inhibitors. However this search had limited success in finding novel class molecules which bear both hydrophobic and hydrophilic moieties.

In continuation of our ongoing report on synthetic inhibitors that targets PLA2[14-15], and other drug-targets[16-22], presently, we herein, report the design of novel drug-like small molecules via chem-informatics approaches followed by synthesis of lead structure, via a retro-synthetic approach as the starting point. Furthermore, we validate the efficacy of these inhibitors against snake venom VRV-PL-VIIIaPLA2 isolated from *vipera russelli* venom and driven by the *in silico* molecular interaction studies, this enabled us to discover novel inhibitors that target PLA2.

RESULTS AND DISCUSSION

MOLPRINT 2D-based identification of drug-like compounds targeting PLA2:

A MOLPRINT-2D PLA2 model was queried with the drug-like molecules of ZINC database [23]. The top ranked compounds are summarized in **Table 1** & **Figure 1**. Among the ranked compounds, ZINC00625534 (DCMB) was ranked 4th. This DCMB was considered as lead molecule as other top ranked compounds failed to contain both hydrophobic and hydrophilic moiety. Since DCMB contain ester linkage, we designed and prepared DCMB analogues.

View Article Online
DOI: 10.1039/C5RA13085A

Synthesis of DCMB analogues:

The lead PLA2 inhibitor ZINC00625534 (DCMB) contains hydrophilic amino, lactone, nitrile, methoxy and ester groups also hydrophobic aromatic rings. To achieve an effectual interaction between sPLA2 active site and an inhibitor, we have replaced the ester group of DCMB by C-C bond which enhances hydrophobicity of the inhibitor. To start with synthesis of DCMB analogues (2-amino chromene-3-carbonitriles), we have employed retro-synthetic approach (Supplementary **Figure S1**) where primary alcohol (**1**) and malanonitrile (**2)** reacts in presence of T3P®-DMSO and ethyl acetate as solvent to give swern oxidized product which further on Knoevenagel condenzation gives product (**3**)[24]. The intermediate **3** undergoes Michael cyclization with 4-hydroxy coumarin (**4**) to form compound **5**. Further, Suzuki coupling reaction of **5** with aromatic and pyridine boronic acids (**6**) where we have used [1,10-bis(diphenylphosphino)ferrocene]dichloro palladium catalyst (Pd(dppf)Cl2) and SCS-Bi_2O_3base in tetrahydrofuran solvent given DCMB analogue 7[25]. The detailed chemical synthesis and characterization of DCMB analogs was presented (**Figure 2**).

Neutralization of snake venom PLA2 by the lead molecule libraries:

In order to test the efficacy of the synthesized inhibitors, they were tested against snake venom PLA2 called VRV-PL-VIIIa isolated from *vipera russelli* venom. Inhibitory effects of the series of lead molecules against PLA2 were tabulated (**Table 2**). All the tested compounds displayed the inhibition of the venom PLA2 with a range from 12 to 68 μM. Among the tested compounds, **7b** showed maximum inhibitory efficacy against PLA2 with an IC_{50} value of 12.5 μM (**Table 3**). Till date, several inhibitors from synthesis, and also from various organisms including marine sponges, snakes, bees, plants

View Article Online
DOI: 10.1039/C5RA13085A

and mammals have been reported. However, this happens to be the first report that the novel chromene molecule, which displayed a potent inhibition of snake venom PLA2.

***In silico* interaction studies of novel ligands that targets PLA2:**

In order to understand structure-based correlation with compound affinity, we conducted molecular docking studies using crystal structure of PLA2 from Russel's viper that bound to nimesulide (PDB: 1ZWP)[26]. The chromene ligands were docked into the PLA2 structure using MOE[27]. We found the cyano functionality of our ligand series consistently replacing the nitro group of nimesulide and forming hydrogen bonds to the backbone nitrogen of Gly-32. The exposed binding site of PLA2 allows for two binding modes of our compounds including these interactions (see **Figure 3**). Both of them form π-π interactions with the readily accessible indole moiety of Trp-31. Different orientation of the core ring system either allows the amine function of the ligands to form hydrogen bonds to the carboxylate of Asp-49 or to the backbone carbonyl of Gly-30. Both predicted binding modes are shown in Figure 3 for the two compounds **7a** and **7b** with highest PLA2 inhibition *in vitro*. Structure-activity relationships within the ligand series are not straightforward to interpret as group-wise contributions since binding modes of the ligands might change and thus give rise to different molecular interactions between PLA2 and the respective compounds.

MATERIALS AND METHODS

***In silico* design of novel small molecule that target PLA2.**

The ligand similarity searching protocol, as implemented in MOLPRINT-2D, was trained using bioactivity data from the ChEMBL database for the target PLA2. Bioactivity training data was extracted from the ChEMBL16 database where activity values (IC50 / EC50 / Ki / Kd) less than

View Article Online
DOI: 10.1039/C5RA13085A

or equal to 10μM, and a ChEMBL confidence score of 8 or greater for 'binding' or 'functional' assays, giving 2,499 active compounds. 1,426 compounds exceeded the 10μM threshold, which were considered to be inactive and used as negative bioactivity training data. MOLPRINT 2D descriptors were generated for the complete data set of active and inactive compounds [28, 29]. The Naïve Bayes learner was subsequently trained on the training compounds and queried with the MOLPRINT 2D fingerprints of 7,228 drug-like compounds of ZINC database. The ZINC molecules were ranked in terms of 'probability of activity' scores generated by models. A 10-fold cross validation with a 50/50 random split of both active and inactive structures was performed, confirming the predictive power of the models.

Chemical Synthesis:

All reagents were commercially available reagent grade were used without further purification. Thin layer chromatography (TLC) was conducted on 0.25 mm silica gel plates ($60F_{254}$, Merck). Column chromatography separations were obtained on silica gel (200-400 mesh). IR spectra were recorded on Bruker FTIR spectrophotometer. ^{1}H NMR spectra were recorded on BrukerAvance-300 instrument in $CDCl_3$ solvent. ^{13}C NMR spectra were obtained on BrukerAvance-300 instrument at 75 MHz in DMSO-d_6 solvent (few on Agilent NMR instrument in $CDCl_3$ solvent). Chemical shifts are expressed in ppm downfield relative to TMS. Mass spectra were recorded on Agilent LC-MS and the elemental analyses were carried out using an Elemental Vario Cube CHNS rapid Analyzer.

General procedure for synthesis of DCMB analogues: To the solvent mixture of ethyl acetate and DMSO (1.5 ml: 0.75ml = 2: 1 ratio) 4-bromo/3-bromo-4-methoxy benzyl alcohol **1** (1.0 mmol) and malanonitrile **2** (1.2 mmol) are added in presence of $T_3P^{®}$ (2.5 mmol, 50% solution in

View Article Online
DOI: 10.1039/C5RA13085A

ethyl acetate) at room temperature, which undergo in-situ Swern oxidation followed by Knoevenagel condensation to yield corresponding alkene **3** within 10 minutes. Complete formation of alkene **3** was confirmed by TLC using hexane:EtOAc (7:3) system and has been observed at R_f 0.78 under ultraviolet (UV) light. Without isolating the alkene **3**, to this reaction mixture 4-hydroxy coumarin **4** (1.0 mmol) was added and stirred for 2-3 hours at room temperature to form compound **5**[30]. Reaction was monitored by TLC (Hexane: EtOAc 7:3) and the compound **5** has been observed at R_f 0.42 under UV light. After completion of the reaction, the mixture was diluted with about 5 ml of distilled water. The product was extracted with 10 ml of ethyl acetate and the combined organic layers were washed with 10 ml of distilled water, followed by 5 ml of brine solution. The organic phase was dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to afford a pure product. Compound **5** was obtained with 98% yield.

Further the compound **5** (1 mmol) heated to 70 ^oC with variety of aryl/hetero boronic acids **6** (1.2 mmol) in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 8-10 hours to obtain crude DCMB analogues (**7 a-n**) (**Table 2**). The formation of final products **7(a-n)** was monitored by TLC (Hexane: EtOAc 8:2). This was further purified by column chromatography using hexane: ethyl acetate as eluent. The final product **7(a-n)** was eluted with 15% hexane:ethyl acetate system (85ml hexane: 15ml ethyl acetate). Thus obtained DCMB analogues were enantiomers and are isolated as recemic mixtures. These DCMB analogues are confirmed by spectral analysis without separation of the recemic mixtures.. Spectral properties were consistent with their assigned structures.

7a 4-([1,1'-biphenyl]-4-yl)-2-amino-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(4-bromophenyl)-5-oxo-4,5-

View Article Online
DOI: 10.1039/C5RA13085A

dihydropyrano[3,2-c]chromene-3-carbonitrile(1 mmol) (compound **5**) and phenyl boronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 8 hours. **7a** has been observed on TLC at R_f 0.58 under UV light and was isolated as white solid by column chromatography with 15% hexane:ethyl acetate system (85ml hexane: 15ml ethyl acetate).

IR ν_{max}: 3323cm^{-1} $\nu_{(NH2)}$, 2194cm^{-1} $\nu_{(CN)}$, 1673cm$^{-1}\nu_{(c-o)}$,1049cm^{-1} $\nu_{(C=O)}$; ^{1}H NMR ($CDCl_3$, 300 MHz):- δ 7.912-7.206 (m, 13H, Ar-H), 4.856 (s, 1H, methine); ^{13}C NMR ($CDCl_3$, 75MHz) δ 161.13, 160.57, 158.53, 156.08, 148.44, 145.95, 134.46, 130.35, 129.75, 128.17, 126.57, 125.46, 124.53, 123.48, 117.66, 112.60, 103.83, 60.20, 35.13; LCMS (MM:ES+APCI) $(M+H)^+$ 393; Anal.Calcd for $C_{25}H_{16}N_2O_3$: C 76.62, H 4.11, N 7.14; Found: C 76.14, H 4.19, N 7.42.

7b 2-amino-4-(2'-methyl-[1,1'-biphenyl]-4-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(4-bromophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile(1 mmol) (compound **5**) and o-tolylboronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 7 hours. **7b** has been observed on TLC at R_f 0.59 under UV light and was isolated as yellow solid by column chromatography with 15% hexane:ethyl acetate system (85ml hexane: 15ml ethyl acetate).

IR ν_{max}: 3256cm^{-1} $\nu_{(NH2)}$, 2196cm^{-1} $\nu_{(CN)}$, 1680cm$^{-1}\nu_{(c-o)}$,1047cm^{-1} $\nu_{(C=O)}$;^{1}H NMR ($CDCl_3$, 300 MHz):- δ 7.831-7.295 (m, 12H, Ar-H), 4.161 (s, 1H, Methine), 3.295 (s, 2H, -NH_2), 2.254 (s, 3H, -CH_3); LCMS (MM:ES+APCI) $(M+H)^+$407; Anal.Calcd for $C_{26}H_{18}N_2O_3$: C 76.83, H 4.46, N 6.89; Found: C 76.91, H 4.53, N 6.81.

View Article Online
DOI: 10.1039/C5RA13085A

7c 2-amino-4-(3'-methoxy-[1,1'-biphenyl]-4-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(4-bromophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile(1 mmol) (compound **5**) and (3-methoxyphenyl)boronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 8 hours. **7c** has been observed on TLC at R_f 0.52 under UV light and was isolated as white solid by column chromatography with 16% hexane:ethyl acetate system (84ml hexane: 16ml ethyl acetate).

IR ν_{max}: 3293cm^{-1} $\nu_{(NH2)}$, 2193cm^{-1} $\nu_{(CN)}$, 1673cm^{-1} $\nu_{(c-o)}$,1048cm^{-1} $\nu_{(C=O)}$; ^{1}H NMR ($CDCl_3$, 300 MHz):- δ 8.000-7.978 (m, 3H, Ar-H), 7.792-7.746 (m, 2H, Ar-H), 7.552-7.521 (m, 2H, Ar-H), 7.434-7.394 (m, 4H, Ar-H), 7.257 (s, 1H, Ar-H), 3.884 (s, 1H, Methine), 3.846 (s, 3H, -OCH_3); ^{13}C NMR (DMSO-D_6, 75MHz) δ 162.52, 160.19, 143.72, 134.25, 131.56, 126.11, 125.87, 125.09, 124.35, 123.46, 122.92, 121.62, 120.95, 120.12, 117.57, 114.29, 112.52, 99.35, 57.28, 52.25, 35.28; LCMS (MM:ES+APCI) $(M+H)^+$ 423; Anal.Calcd for $C_{26}H_{18}N_2O_4$: C 73.92, H 4.29, N 6.63; Found: C 73.88, H 4.32, N 6.59.

7d 2-amino-5-oxo-4-(2'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(4-bromophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile(1 mmol) (compound **5**) and (2-(trifluoromethyl)phenyl)boronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 9 hours. **7d** has been observed on TLC at R_f 0.50 under UV light and was isolated as grey solid by column chromatography with 16% hexane:ethyl acetate system (84ml hexane: 16ml ethyl acetate).

View Article Online
DOI: 10.1039/C5RA13085A

IR ν_{max}: 3292cm^{-1} $\nu_{(NH2)}$, 2199cm^{-1} $\nu_{(CN)}$, 1669cm^{-1} $\nu_{(c\text{-}o)}$,1033cm^{-1} $\nu_{(C=O)}$; ^{1}H NMR ($CDCl_3$, 300 MHz):- δ 7.831-7.260 (m, 12H, Ar-H), 4.714 (s, 1H, Methine); ^{13}C NMR ($CDCl_3$, 75MHz) δ 162.57, 161.43, 159.27, 158.12, 143.29, 141.25, 135.04, 132.81, 128.77, 126.92, 126.03, 125.12, 124.69, 123.14, 121.20, 120.07, 118.77, 116.83, 110.32, 100.05, 58.23, 38.48; LCMS (MM:ES+APCI) $(M+H)^+$ 461; Anal.Calcd for $C_{26}H_{15}F_3N_2O_3$: C 67.83, H 3.28, N 12.38; Found: C 67.90, H 3.31, N 12.41.

7e 2-amino-4-(4'-chloro-3'-(trifluoromethyl)-[1,1'-biphenyl]-4-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(4-bromophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile(1 mmol) (compound **5**) and (4-chloro-3-(trifluoromethyl)phenyl)boronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 8.5 hours. **7e** has been observed on TLC at R_f 0.50 under UV light and was isolated as white solid by column chromatography with 16% hexane:ethyl acetate system (84ml hexane: 16ml ethyl acetate).

IR ν_{max}: 3293cm^{-1} $\nu_{(NH2)}$, 2200cm^{-1} $\nu_{(CN)}$, 1667cm^{-1} $\nu_{(c\text{-}o)}$,1050cm^{-1} $\nu_{(C=O)}$; ^{1}H NMR ($CDCl_3$, 300 MHz):- δ 7.842-7.260 (m, 11H, Ar-H), 4.851 (s, 1H, Methine); ^{13}C NMR ($CDCl_3$, 75MHz) δ 161.08, 160.50, 158.19, 141.89, 135.08, 132.50, 130.19, 128.11, 127.47, 126.09, 124.05, 123.74, 122.29, 121.08, 119.85, 117.64, 115.24, 111.48, 101.56, 60.15, 36.22; LCMS (MM:ES+APCI) $(M-H)^-$ 493; Anal.Calcd for $C_{26}H_{14}ClF_3N_2O_3$: C 63.11, H 2.85, N 5.66; Found: C 63.15, H 2.89, N 5.70.

7f 2-amino-5-oxo-4-(4-(pyridin-3-yl)phenyl)-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(4-bromophenyl)-5-oxo-4,5-

View Article Online
DOI: 10.1039/C5RA13085A

dihydropyrano[3,2-c]chromene-3-carbonitrile(1 mmol) (compound **5**) and pyridin-3-ylboronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 8.5 hours. **7f** has been observed on TLC at R_f 0.55 under UV light and was isolated as white solid by column chromatography with 15% hexane:ethyl acetate system (85ml hexane: 15ml ethyl acetate).

IR ν_{max}: 3323cm^{-1} $\nu_{(NH2)}$, 2195cm^{-1} $\nu_{(CN)}$, 1668cm$^{-1}\nu_{(c-o)}$,1042cm^{-1} $\nu_{(C=O)}$;^{1}H NMR ($CDCl_3$, 300 MHz):- δ 8.922-8.918 (s, 1H, Ar-N-CH), 8.688-8.673 (d, 1H, Ar-H), 8.568-8.548 (d, 1H, Ar-H), 7.996-7.961 (m, 2H, Ar-H), 7.869-7.846 (m, 1H, Ar-H), 7.586-7.589 (m, 3H, Ar-H), 7.428-7.370 (m, 3H, Ar-H), 4.747 (s, 1H, Methine), 1.566 (s, 2H, -NH_2); LCMS (MM:ES+APCI) $(M+H)^{+}$394; Anal.Calcd for $C_{24}H_{15}N_3O_3$: C 73.27, H 3.84, N 10.68; Found: C 73.31; H, 3.88; N, 10.73.

7g 2-amino-4-(4-(5,6-dichloropyridin-3-yl)phenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(4-bromophenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile(1 mmol) (compound **5**) and (4,6-dichloropyridin-3-yl)boronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 9.5 hours. **7g** has been observed on TLC at R_f 0.51 under UV light and was isolated as white solid by column chromatography with 16% hexane:ethyl acetate system (84ml hexane: 16ml ethyl acetate).

IR ν_{max}: 3320cm^{-1} $\nu_{(NH2)}$, 2192cm^{-1} $\nu_{(CN)}$, 1665cm$^{-1}\nu_{(c-o)}$,1046cm^{-1} $\nu_{(C=O)}$; ^{1}H NMR ($CDCl_3$, 300 MHz):- δ 8.625 (s, 1H, Ar-N-H), 8.462 (s, 1H, Ar-H), 7.772 (m, 1H, Ar-H), 7.595-7.501 (m, 1H, Ar-H), 7.386-7.324 (m, 2H, Ar-H), 7.003-6.896 (m, 3H, Ar-H), 3.725 (s, 1H, Methine); LCMS

View Article Online
DOI: 10.1039/C5RA13085A

(MM:ES+APCI) $(M+H)^+$ 463; Anal.Calcd for $C_{24}H_{13}Cl_2N_3O_3$: C 62.35, H 2.83, N 9.09; Found: C 62.31, H 2.79, N 9.12.

7h 2-amino-4-(6-methoxy-[1,1'-biphenyl]-3-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(3-bromo-4-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (1 mmol) (compound **5**) and phenyl boronic acid **6**(1.2 mmol) at 70 ^oC in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 8 hours. **7h** has been observed on TLC at R_f 0.56 under UV light and was isolated as yellow solid by column chromatography with 15% hexane:ethyl acetate system (85ml hexane: 15ml ethyl acetate).

IR ν_{max}:3286cm^{-1} $\nu_{(NH2)}$, 2197cm^{-1} $\nu_{(CN)}$, 1667$cm^{-1}$$\nu_{(c-o)}$,1052$cm^{-1}$ $\nu_{(C=O)}$; ^1H NMR ($CDCl_3$, 300 MHz):- δ 7.797-7.776 (s, 1H, Ar-H), 7.772-7.7.52 (m, 1H, Ar-H), 7.491-7.467 (m, 2H, Ar-H), 7.374-7.336 (m, 6H, Ar-H), 7.192-7.186 (m, 1H, Ar-H), 6.939-.917 (m, 1H, Ar-H), 3.867 (s, 1H, Methine), 3.750-3.715 (s, 3H, $-OCH_3$); LCMS (MM:ES+APCI) $(M-H)^-$ 421; Anal.Calcd for $C_{26}H_{18}N_2O_4$: C 73.92, H 4.29, N 6.63; Found: C 73.88, H4.26, N 6.67.

7i 2-amino-4-(6-methoxy-2'-methyl-[1,1'-biphenyl]-3-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(3-bromo-4-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (1 mmol) (compound **5**) and o-tolylboronic acid **6**(1.2 mmol) at 70 ^oC in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 8.5 hours. **7i** has been observed on TLC at R_f 0.57 under UV light and was isolated as white solid by column chromatography with 15% hexane:ethyl acetate system (85ml hexane: 15ml ethyl acetate).

View Article Online
DOI: 10.1039/C5RA13085A

IR ν_{max}: 3288cm^{-1} $\nu_{(NH2)}$, 2196cm^{-1} $\nu_{(CN)}$, 1668cm$^{-1}\nu_{(c-o)}$,1057cm^{-1} $\nu_{(C=O)}$; ^{1}H NMR ($CDCl_3$, 300 MHz):- δ 7.775-7.758 (m, 1H, Ar-H), 7.356-7.348 (m, 3H, Ar-H), 7.260 (m, 3H, Ar-H), 7.030-6.902 (m, 4H, Ar-H), 4.629 (1H, Methine, s), 3.734 (3H, -OCH_3, s), 2.069 (3H, -CH_3, s); LCMS (MM:ES+APCI) $(M-H)^-$ 435; Anal.Calcd for $C_{27}H_{20}N_2O_4$: C 74.30, H 4.62, N 6.42; Found: C74.28, H 4.65, N 6.46.

7j 2-amino-4-(3',6-dimethoxy-[1,1'-biphenyl]-3-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(3-bromo-4-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile(1 mmol) (compound **5**) and (3-methoxyphenyl)boronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 9.5 hours. **7j** has been observed on TLC at R_f 0.50 under UV light and was isolated as brown solid by column chromatography with 16% hexane:ethyl acetate system (84ml hexane: 16ml ethyl acetate).

IR ν_{max}: 3287cm^{-1} $\nu_{(NH2)}$, 2197cm^{-1} $\nu_{(CN)}$, 1669cm^{-1} $\nu_{(c-o)}$,1053cm^{-1} $\nu_{(C=O)}$; ^{1}H NMR ($CDCl_3$, 300 MHz):- δ 7.791-7.773 (s, 1H, Ar-H), 7.613-7.574 (m, 2H, Ar-H), 7.354-7.335 (m, 3H, Ar-H), 7.260 (m, 1H, Ar-H), 7.065-7.037 (m, 2H, Ar-H), 6.936-6.847 (m, 2H, Ar-H), 6.847 (m, 11H, Ar-H), 3.811 (s, 1H, Methine), 3.779 (s, 6H -OCH_3); ^{13}C NMR (DMSO-D_6, 75MHz) δ 161.52, 160.25, 154.12, 152.74, 132.13, 126.98, 126.17, 125.01, 124.45, 123.85, 123.11, 122.92, 121.51, 119.25, 116.52, 113.32, 110.59, 100.50, 60.15, 56.72, 55.91, 36.71; LCMS (MM:ES+APCI) $(M-H)^-$ 451; Anal.Calcd for $C_{27}H_{20}N_2O_5$: C 71.67, H 4.46, N 6.19; Found: C 71.71, H 4.41, N 6.21.

7k 2-amino-4-(6-methoxy-2'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-

View Article Online
DOI: 10.1039/C5RA13085A

amino-4-(3-bromo-4-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (1 mmol) (compound **5**) and (2-(trifluoromethyl)phenyl)boronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 8.2 hours. **7k** has been observed on TLC at R_f 0.48 under UV light and was isolated as brown solid by column chromatography with 17% hexane:ethyl acetate system (83ml hexane: 17ml ethyl acetate).

IR ν_{max}: 3301cm^{-1} $\nu_{(NH2)}$, 2198cm^{-1} $\nu_{(CN)}$, 1671cm^{-1} $\nu_{(c-o)}$,1053cm^{-1} $\nu_{(C=O)}$;^{1}H NMR ($CDCl_3$, 300 MHz):- δ 7.925-7.885 (m, 2H, Ar-H), 7.815 (s, 1H, Ar-H), 7.651-7.552 (m, 2H, Ar-H), 7.450-7.321 (m, 3H, Ar-H), 7.250 (m, 2H, Ar-H), 6.982 (s, 1H, Ar-H), 4.297 (s, 1H, Methine), 3.306 (s, 3H, -OCH_3); ^{13}C NMR ($CDCl_3$, 75MHz) δ 162.19, 161.28, 159.14, 158.34, 133.65, 131.28, 129.52, 125.87, 125.19, 124.57, 124.01, 123.85, 123.08, 122.12, 117.12, 115.28, 103.52, 59.28, 55.71, 36.17;LCMS (MM:ES+APCI) $(M+H)^+$ 491; Anal.Calcd for $C_{27}H_{17}F_3N_2O_4$: C 66.12, H 3.49, N 5.71; Found: C 66.13, H 3.52, N 5.73.

7l 2-amino-4-(4'-chloro-6-methoxy-3'-(trifluoromethyl)-[1,1'-biphenyl]-3-yl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(3-bromo-4-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (1 mmol) (compound **5**) and (4-chloro-3-(trifluoromethyl)phenyl)boronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 9 hours. **7l** has been observed on TLC at R_f 0.47 under UV light and was isolated as white solid by column chromatography with 17% hexane:ethyl acetate system (83ml hexane: 17ml ethyl acetate).

View Article Online
DOI: 10.1039/C5RA13085A

IR ν_{max}: 3288cm^{-1} $\nu_{(NH2)}$, 2198cm^{-1} $\nu_{(CN)}$, 1671cm^{-1} $\nu_{(c\text{-}o)}$,1055cm^{-1} $\nu_{(C=O)}$; ^{1}H NMR ($CDCl_3$, 300 MHz):- δ 7.819-7.799 (d, 1H, Ar-H), 7.638-7.616 (m, 2H, Ar-H), 7.599-7.571 (m, 2H, Ar-H), 7.504 (m, 2H, Ar-H), 7.392-7.510 (m, 2H, Ar-H), 6.869-6.848 (s, 1H, Ar-H), δ 3.870 (s, 1H, Methine), δ 3.787 (s, 3H -OCH_3); ^{13}C NMR (DMSO-D_6, 75MHz) δ 161.57, 160.16, 158.91, 144.15, 135.46, 133.48, 132.19, 125.45, 124.89, 124.25, 123.10, 122.59, 121.85, 121.41, 119.32, 116.28, 114.11, 113.42, 101.41, 59.12, 55.72, 36.29; LCMS (MM:ES+APCI) (M-H)$^-$ 523; Anal.Calcd for $C_{27}H_{16}ClF_3N_2O_4$: C 61.78, H 3.07, N 5.34; Found: C 61.74, H 3.05, N 5.30.

7m 2-amino-4-(4-methoxy-3-(pyridin-3-yl)phenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-amino-4-(3-bromo-4-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile(1 mmol) (compound **5**) and pyridin-3-ylboronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 8 hours. **7m** has been observed on TLC at R_f 0.51 under UV light and was isolated as white solid by column chromatography with 16% hexane:ethyl acetate system (84ml hexane: 16ml ethyl acetate).

IR ν_{max}: 3292cm^{-1} $\nu_{(NH2)}$, 2199cm^{-1} $\nu_{(CN)}$, 1674cm^{-1} ^{1} $\nu_{(c\text{-}o)}$,1066cm^{-1} $\nu_{(C=O)}$; ^{1}H NMR ($CDCl_3$, 300 MHz):- δ 8.718-8.672 (s, 1H, Ar-H), 8.608-8.511 (s, 1H, Ar-H), 8.112-7.941 (m, 2H, Ar-H), 7.819-7.766 (m, 1H, Ar-H), 7.606-7.588 (m, 2H, Ar-H), 7.518-7.310 (m, 4H, Ar-H), 4.627 (s, 1H, Methine), 3.656 (s, 3H -OCH_3) LCMS (MM:ES+APCI) (M-H)$^-$ 422; Anal.Calcd for $C_{25}H_{17}N_3O_4$: C 70.91, H 4.05, N 9.92; Found: C 70.89, H 4.09, N 9.90.

7n 2-amino-4-(3-(4,6-dichloropyridin-3-yl)-4-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile: This compound was obtained by heating 2-

View Article Online
DOI: 10.1039/C5RA13085A

amino-4-(3-bromo-4-methoxyphenyl)-5-oxo-4,5-dihydropyrano[3,2-c]chromene-3-carbonitrile (1 mmol) (compound **5**) and (4,6-dichloropyridin-3-yl)boronic acid **6**(1.2 mmol) at 70 ^{o}C in presence of $Pd(dppf)_2Cl_2$ catalyst (0.001 mmol), SCS—Bi_2O_3 (0.5 mmol) base in 1 ml water and 4 ml of tetrahydrofuran solvent for 8.5 hours. **7n** has been observed on TLC at R_f 0.48 under UV light and was isolated as brown solid by column chromatography with 16% hexane:ethyl acetate system (84ml hexane: 16ml ethyl acetate).

IR ν_{max}: 3228cm^{-1} $\nu_{(NH2)}$, 2193 cm^{-1} $\nu_{(CN)}$, 1671cm^{-1}$\nu_{(c-o)}$,1051cm^{-1} $\nu_{(C=O)}$; ^{1}H NMR ($CDCl_3$, 300 MHz):- δ 8.601 (s, 1H, Ar-N-CH), 7.716-7.182 (m, 8H, Ar-H), 4.112 (s, 1H, Methine), 3.603 (s, 3H, -OCH_3); LCMS (MM:ES+APCI) $(M+H)^+$ 493; Anal.Calcd for $C_{25}H_{15}Cl_2N_3O_4$: C 60.99, H 3.07, N 8.54; Found: C 60.96, H 3.05, N 8.59.

Substrate preparation: Briefly, a working solution of 1mM DMPC (Substrate) was prepared in methanol containing 2mM Triton X-100 in Milli-Q water. The resulting substrate solution was spun at 1,500xg for 5min to form uniform mixed micelles.

LPC standardcurve: LPC (1-myristoyl-2-hydroxy-*Sn*-glycerol-3-phosphocholine) standard curve was constructed using different LPC concentrations ranging from 0 to 100µM as reported earlier[31]. Briefly, total reaction mixture of 100µL containing activity buffer (50mM Tris, pH 7.5, 10mM $CaCl_2$) and 2mM LPC containing 4mM Triton X-100 (1:2 ratio) and incubated for 5 min at 37ºC. Quenching solution was added and vortexed for 30 Sec and incubated for 5 min at RT. 2µL of reaction mixture was pipetted to measure RFU as described earlier.

Neutralization of VRV-PL-VIIIa (sPLA2) by the title compounds: sPLA2 activity was assayed according to the method[32]. Briefly, a 50µl activity buffer containing 50mM Tris-HCl buffer pH 7.5, 10mM $CaCl_2$ and 10µl substrate stock were added and incubated for 5 min at 37ºC. Activity was initiated by adding 10ng of sPLA2 alone or pre incubated with different

View Article Online
DOI: 10.1039/C5RA13085A

concentration of DCMB analogues ranging from 0-120µM for 5min at 37°C. Reaction mixture was incubated for 45 min at 37ºC. 50µl of quenching solution was added at final concentration of 2mM NaN_3, 50µM ANS and 50mM EGTA, vortexed for 30 sec and incubated for 5 min at RT. 2µl of this solution was pipetted to measure Relative fluorescence unit (RFU) in a Nano drop ND3300 Ver 2.8 using excitation with UV-LED (370 ±10nm) and emission was recorded at 480nm in dark condition. Enzyme activity was calculated by Eq (1), where ΔRFU is the change in RFU of test (with sPLA2) with respect to control (without sPLA2) in presence of inhibitor. The resultant RFU is compared with standard curve of LPC to determine the sPLA2 activity in presence of inhibitor. 4^{th} parameter logical (4PL) fit module of Graphpad Prism 6.05 was used to compute IC_{50} values.

Equation 1

$$\mathbf{\Delta RFU_{LPC} = RFU_c - RFU_t}$$

Molecular Docking Studies: We docked the series of fourteen synthesized compounds to the crystal structure of PLA2 from Russel's viper in complex with nimesulide (PDB: 1ZWP). We used identical settings as in an earlier study on imidazopyridine-based PLA2inhibitors docking in MOE. The protocol included a pharmacophore filter during docking to enforce a hydrogen bond acceptor feature in the position of the nitro group of nimesulide. Predicted binding modes were visualized in Pymol[33-35].

CONCLUSION

In conclusion, we herein report a simple, efficient, catalyst free and one potsynthetic route to prepare tri-substituted-condensed-imidazopyridines and our*in silico* target prediction presented PLA2 as a likely target for the newly synthesized compounds. The prediction was experimentally validated using indirect haemolytic assay. Of the new compounds

View Article Online
DOI: 10.1039/C5RA13085A

synthesized, 1-(2-Methyl-8-naphthalen-1-yl-imidazo [1,2-α]pyridine-3-yl)-ethanone was identified as the lead compound with an IC_{50} value of 14.3 μM. Molecular docking analysis displayed that the imidazopyridine compounds could make a favourable π-π stacking interactions with Trp-31. Exploration of PLA2 inhibitory activity of imidazopyridine derivatives contributes to the development of the title compounds as therapeutic agents to block the PLA2 associated inflammatory diseases. Thus, synthesis of more imidazopyridine derivatives and optimization of their biological activity according to the identified structure-activity relationship is envisaged.

Acknowledgements

This research was supported by University Grants Commission (41-257-2012-SR), Vision Group Science and Technology, Department of Science and Technology (NO. SR/FT/LS-142/2012). Keerthy HK, Bharathkumar H thanks to University Grants Commission for Basic Science Research Fellowship. Andreas Bender thanks Unilever and the European Research Commission (ERC Starting Grant 2013) for funding. Lewis Mervin thanks the BBSRC and AstraZeneca for funding.

View Article Online
DOI: 10.1039/C5RA13085A

References

1. JE Burke, Dennis E. A, *J Lipid Res,* **2009**, 50, S237-42.
2. M. Murakami, I. Kudo, *J Biochem,* **2002**, 131, 285-92.
3. I. Kudo, M. Murakami, *Prostaglandins Other Lipid Mediat*, **2002**, 68, 3-58.
4. R. P. Samy, P. Gopalakrishnakone, B. G. Stiles, K. S. Girish, S. N. Swamy, M. Hemshekhar, K. S. Tan, E. G. Rowan, G. Sethi, V. T. Chow, *Curr Med Chem*, **2012**, 19, 6150-62.
5. L. Perrin-Cocon, S. Agaugue, F. Coutant, A. Masurel, S. Bezzine, G. Lambeau, P. Andre, V. Lotteau, *Eur J Immunol*, **2004**, 34, 2293–02.
6. Moura-da-Silva AM1, Paine MJ, Diniz MR, Theakston RD, Crampton JM, *J Mol Evol*, **1995**, 41, 174-9.
7. Sato H, Isogai Y, Masuda S, Taketomi Y, Miki Y, Kamei D, Hara S, Kobayashi T, Ishikawa Y, Ishii T, Ikeda K, Taguchi R, Ishimoto Y, Suzuki N, Yokota Y, Hanasaki K, Suzuki- Yamamoto T, Yamamoto K, Murakami M, *J BiolChem,* **2011**, 286, 11632-48.
8. Venkatesan C, Sarathi M, Balasubramanian G, Thomas J, Balachander V, Babu VS, Bilal SM, Majeed SA, Madan N, Raj NS, Vimal S, Nambi KS, Hameed AS, *Hum Exp Toxicol,* **2014**, 33, 336-59.
9. E. Valentin, G. Lambeau, *Biochimie*, **2000**, 82, 815–831.
10. R. K. Arni, R. J. Ward, *Toxicon,* **1996**, 34, 827–41.
11. L¨attig, M. B¨ohl, P. Fischer et al., *Journal of Computer-Aided Molecular Design*, **2007**, 21, 473–483.
12. T. Wei-Yi Ong, G. Farooqui, A. A. Kokotos, Farooqui, *ACS Chem. Neurosci,* **2015**, just accepted manuscript.

View Article Online
DOI: 10.1039/C5RA13085A

13. Marcussi S, Sant'Ana CD, Oliveira CZ, Rueda AQ, Menaldo DL, Beleboni RO, Stabeli RG, Giglio JR, Fontes MR, Soares AM, *Curr Top Med Chem*, **2007**, 7, 743-56.

14. Anilkumar NC, Sundaram MS, Mohan CD, Rangappa S, Bulusu KC, Fuchs JE, Girish KS, Bender A, Basappa, Rangappa KS. *PLoS One*. 2015, 10, e0131896.

15. Basappa, Satish Kumar M, Nanjunda Swamy S, Mahendra M, Shashidhara Prasad J, Viswanath BS, Rangappa KS. *Bioorg Med Chem Lett.* 2004, 14, 3679-81.

16. Sukhorukov AY, Nirvanappa AC, Swamy J, Ioffe SL, Nanjunda Swamy S, Basappa, Rangappa KS. *Bioorg Med Chem Lett.* 2014, 24, 3618-21.

17. Chandramohanadas R, Basappa, Russell B, Liew K, Yau YH, Chong A, Liu M, Gunalan K, Raman R, Renia L, Nosten F, Shochat SG, Dao M, Sasisekharan R, Suresh S, Preiser P. J Infect Dis. 2014, 210, 1616-26.

18. Neelgundmath M, Dinesh KR, Mohan CD, Li F, Dai X, Siveen KS, Paricharak S, Mason DJ, Fuchs JE, Sethi G, Bender A, Rangappa KS, Kotresh O, Basappa. *Bioorg Med Chem Lett*. 2015, 25, 893-7.

19. Priya BS, Anil Kumar C, Nanjunda Swamy S, Basappa, Naveen S, Shashidhara Prasad J, Rangappa KS. *Bioorg Med Chem Lett.* 2007, 17, 2775-80.

20. Priya BS, Swamy SN, Tejesvi MV, Basappa, Sarala G, Gaonkar SL, Naveen S, Prasad JS, Rangappa KS. *Eur J Med Chem.* 2006, 41, 1262-70.

21. Sadashiva MP, Basappa S, Nanjundaswamy S, Li F, Manu KA, Sengottuvelan M, Prasanna DS, Anilkumar NC, Sethi G, Sugahara K, Rangappa KS. *BMC Chem Biol*. 2012, 12, 5.

22. Li F, Yamada S, Basappa, Shetty AK, Sugiura M, Sugahara K. *Glycoconj J.* 2008, 25, 603-10.

View Article Online
DOI: 10.1039/C5RA13085A

23. John J. Irwin, Teague Sterling, Michael M. Mysinger, Erin S. Bolstad, and Ryan G. Coleman, *J Chem Inf Model*, **2012,** 52, 1757–68.

24. Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, Davies M, Kruger FA, Light Y, Mak L, McGlinchey S, Nowotka M, Papadatos G, Santos R, Overington JP. *Nucleic Acids Res.* **2014**, 42, 1031.

25. S. Anusha, B. S. Anandakumar, C. D. Mohan, G. P. Nagabhushana, B. S. Priya, K. S. Rangappa, Basappa, G. T. Chandrappa, *RSC Adv*, **2014**, 4, 52181-88.

26. Singh N, Kumar RP, Kumar S, Sharma S, Mir R, et al. *J Mol Recognit*, **2009**, 22, 437-445.

27. *Molecular Operating Environment* (MOE) CCGI, Montreal Q, Canada, **2013**.

28. P. Willett, J.M. Barnard and G.M. Downs. *J Chem Inf Comp Sci,* **1998**, 38, 983-96.

29. A. Bender and R.C. Glen. *Organic and Biomolecular Chemistry*, **2004**, 2, 3204-18.

30. Keerthy HK, Garg M, Mohan CD, Madan V, Kanojia D, Shobith R, Nanjundaswamy S, Mason DJ, Bender A, Basappa, Rangappa KS, Koeffler HP, *PLoS One*, **2014**, 9, e107118.

31. Rangappa, K. S. *Current Topics in Medicinal Chemistry*, **2007**, 7, 741-742.

32. H. K. Vivek, S. G. Swamy, B. S. Priya, G. Sethi, K. S. Rangappa, S. N. Swamy, Anal Biochem, **2014**, 461, 27.35.

33. Bender A, Mussa HY, Glen RC, Reiling S, *J Chem Inf Compute Sci,* **2004**, 44, 1708-18.

34. Bender A, Mussa HY, Glen RC, *J Biomol Screen,* **2005**, 10, 658-66.

35. De Lano W: The Pymol Molecular Graphics System v, De Lano Scientific, San Carlos, CA.

View Article Online
DOI: 10.1039/C5RA13085A

Figure 1: Structural representation of *in silico* ranked structure ZINC0062553 (DCMB) and its analogues.

Figure 2: Synthesis scheme for DCMB analogues 7(a-n).

Figure 3: Molecular interaction studies of novel ligands that targets PLA2. Predicted molecular interactions between PLA2 and DCMB analogues: PLA2 is shown as grey cartoon with semi-transparent surface representation. Main interaction centres Asp-49, Gly-32, Trp-31, and Gly-30 (from left to right) are highlighted as lines in atomic colouring. A) Binding mode of nimesulide within the co-crystal structure used for docking (PDB: 1ZWP, [4]). **a** and **7b** are predicted to bind in two different modes to PLA2. Both of them replace the nitro group of nimesulide by a cyano group and show π-π interactions with Trp-31 but form different molecular interactions via the amine group (7a: Asp-49, 7b: Gly-30).

View Article Online
DOI: 10.1039/C5RA13085A

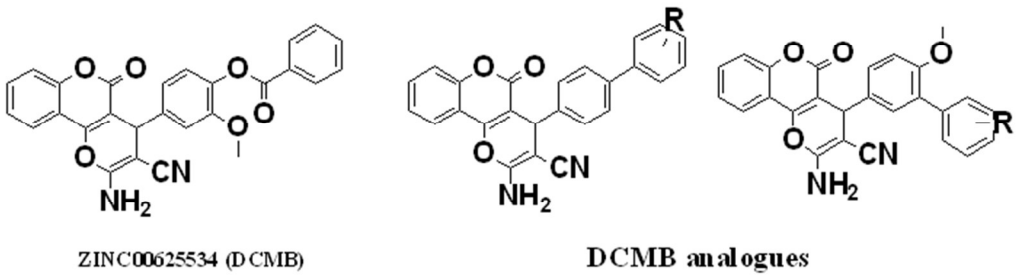


Figure 1

View Article Online
DOI: 10.1039/C5RA13085A

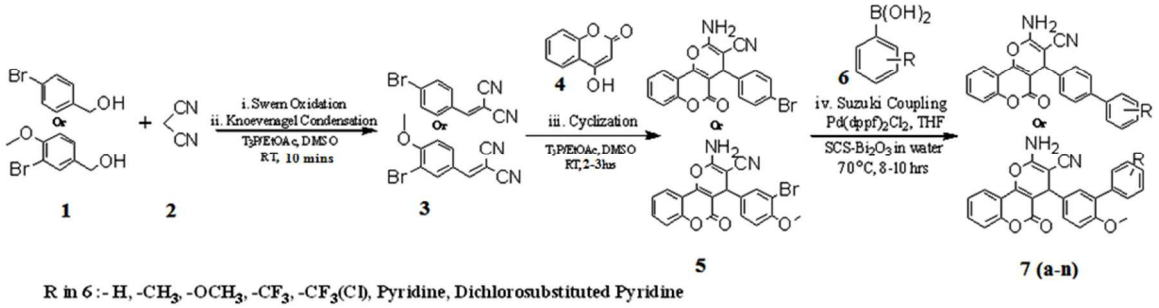


Figure 2

View Article Online
DOI: 10.1039/C5RA13085A

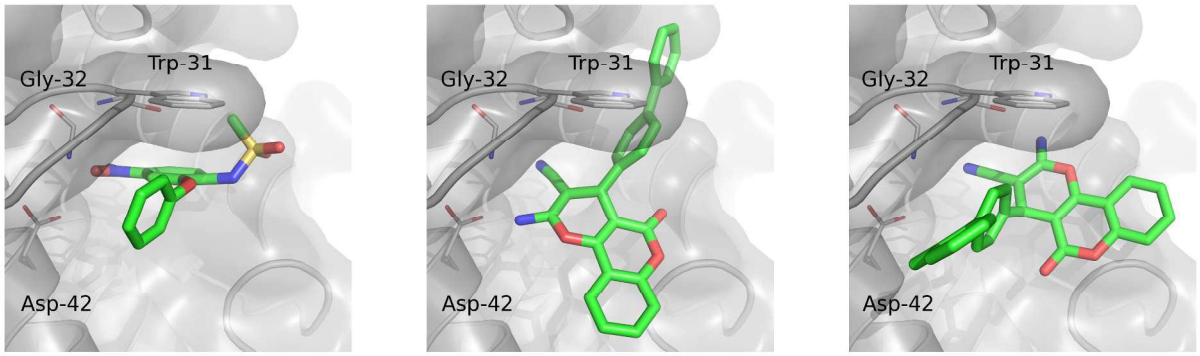
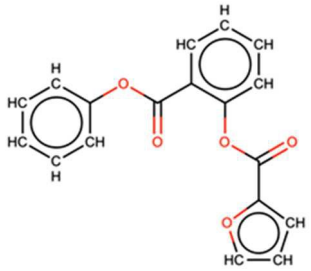
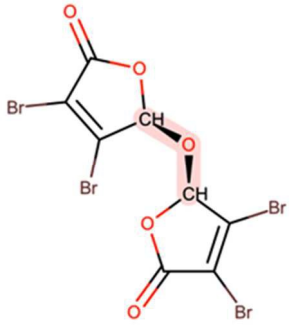
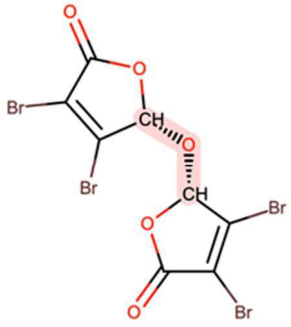


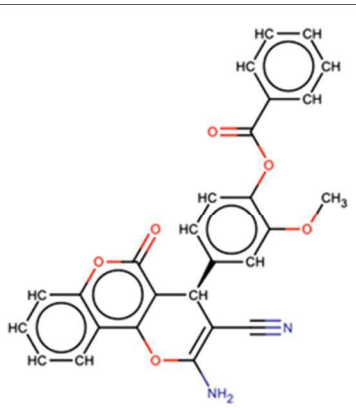
Figure 3

View Article Online
DOI: 10.1039/C5RA13085A

Table 1: List of ranked compounds targeting PLA2 based on the designing of MOLPRINT 2D.

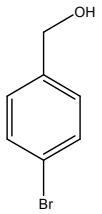
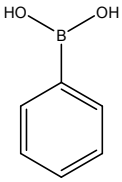
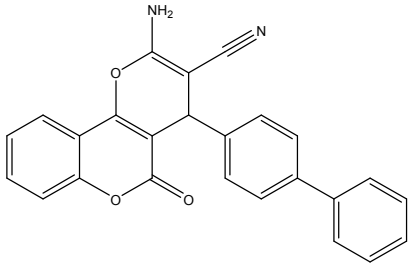
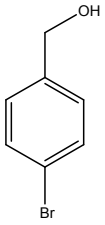
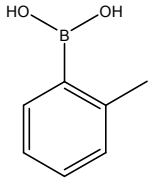
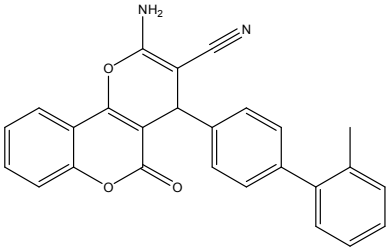
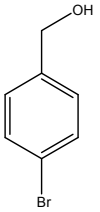
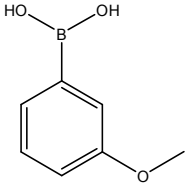
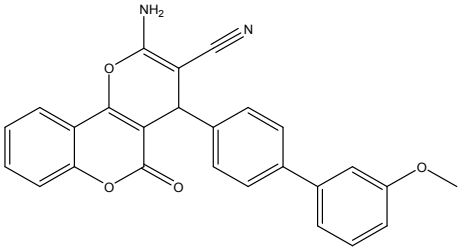
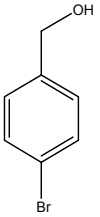
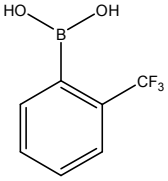
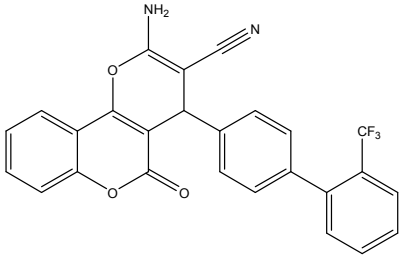
Rank	Zinc Accession	Compound name	Structure	Probability of Activity
1	ZINC00299345	2-(phenoxycarbonyl)phenyl 2-furoate		2531.0
2	ZINC08427108	(5R,5'R)-5,5'-oxybis(3,4-dibromofuran-2(5H)-one)		2295.8
3	ZINC08427105	(5S,5'S)-5,5'-oxybis(3,4-dibromofuran-2(5H)-one)		2295.8

View Article Online
DOI: 10.1039/C5RA13085A

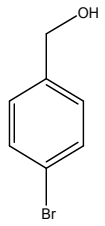
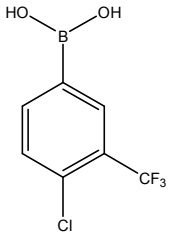
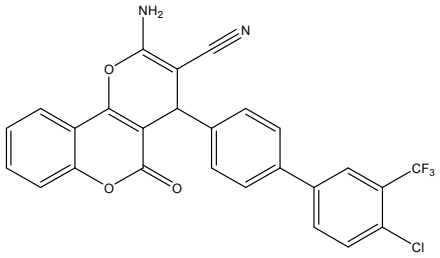
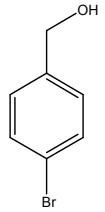
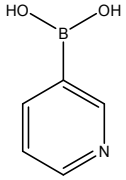
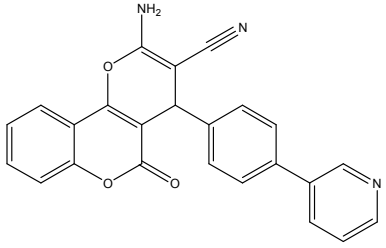
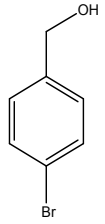
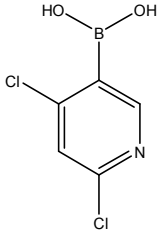
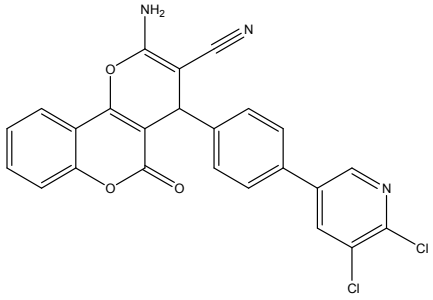
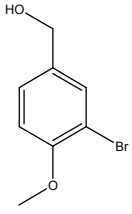
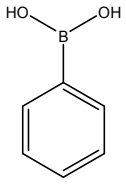
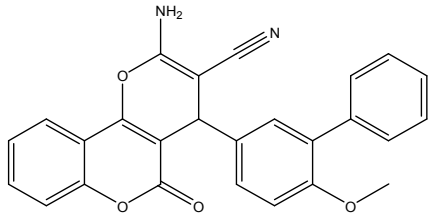
4	ZINC00625534	4-(2-amino-3-cyano-5-oxo-4H,5H-pyrano[3,2-c]chromen-4-yl)-2-methoxyphenyl benzoate		1819.7

View Article Online
DOI: 10.1039/C5RA13085A

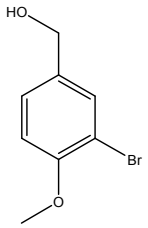
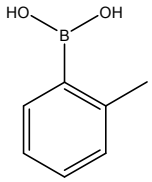
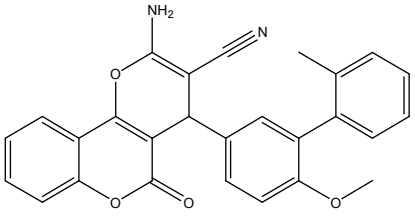
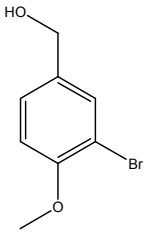
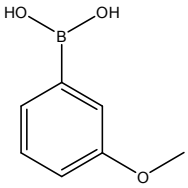
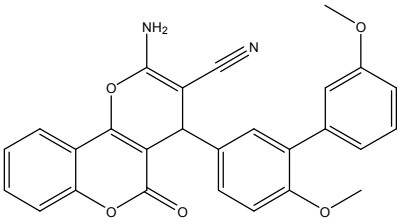
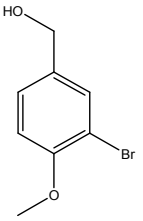
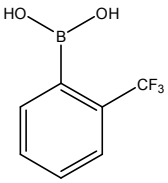
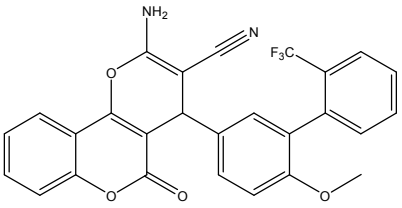
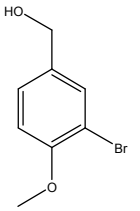
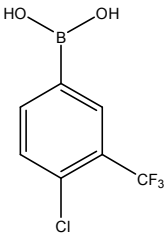
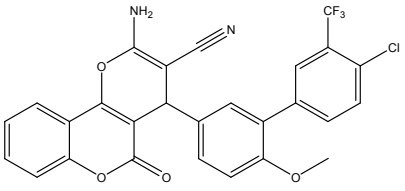
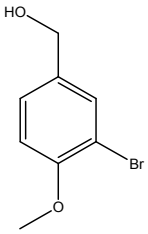
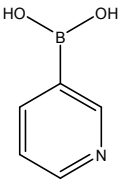
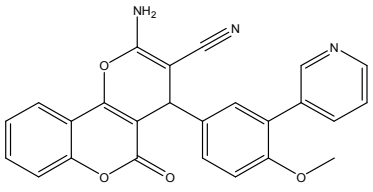
Table2: Newly synthesized DCMB analogues

Entry	1	6	7(a-n)	[a]Yield%
1			 **7a**	92
2			 **7b**	90
3			 **7c**	89
4			 **7d**	84

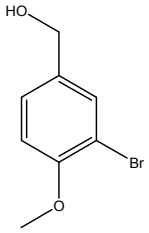
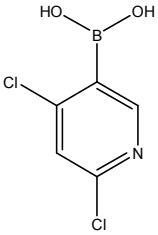
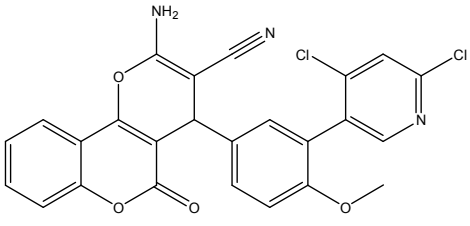
View Article Online
DOI: 10.1039/C5RA13085A

5			 **7e**	85
6			 **7f**	89
7			 **7g**	93
8			 **7h**	91

View Article Online
DOI: 10.1039/C5RA13085A

9			 **7i**	92
10			 **7j**	90
11			 **7k**	84
12			 **7l**	87
13			 **7m**	86

View Article Online
DOI: 10.1039/C5RA13085A

14				83
			7n	

[a] Isolated

View Article Online
DOI: 10.1039/C5RA13085A

Table 3: *In-vitro* inhibition of PLA2 by DCMB analogues.

Sl. No	Ligands	IC_{50} (μM)
1	**7a**	13.61μM
2	**7b**	12.50μM
3	**7c**	22.67μM
4	**7d**	ND[a]
5	**7e**	ND
6	**7f**	40.35μM
7	**7g**	63.85μM
8	**7h**	Inactive
9	**7i**	57.03μM
10	**7j**	18.95μM
11	**7k**	ND
12	**7l**	ND
13	**7m**	20.54μM
14	**7n**	18.94μM

[a]ND, not determined.